BIOPRESERVATION TOOLS FOR CELLS, TISSUES, AND ORGANS

Process Protocol for Cell Isolation from Vascularized Tissues

I. Tissue Preparation Procedure at harvest site (at room temperature)
   A. To prepare the cells in the organ for cold environment, perfuse or flush the tissue with room temperature (18-22°C) PrepaStor® to remove native fluids from the tissue (i.e. blood).
   B. Re-perfuse the tissue with cold (2-8°C) HypoThermosol® FRS (HTS-FRS) to replace the PrepaStor® in the tissue for cold storage.
   C. Bathe the tissue in cold (2-8°C) HTS-FRS for storage and transportation.
   D. Transportation and storage of the organ should be done under hypothermic conditions (2-8°C).

II. Cell Harvest (Standard Practice)
   A. Remove tissue from storage; perfuse the tissue with PrepaStor to remove the preservation solution.
   B. Perfuse tissue with digestion cocktail solution.
   C. Dissociate the tissue into isolated cells.
   D. Screen sample to purify cell isolates.
   E. Centrifuge samples to collect viable cells.
   F. Re-suspend
      i. Re-suspend as necessary in culture media (for cell culture)
      ii. Re-suspend as necessary in HTS-FRS (for suspended cell short term storage; hypothermic storage)
         a. If cells are being plated for culture and subsequent utilization, plates can be placed into hypothermic storage (2-8°C) after a day or two of culture for short-term storage. This allows for an expanded window of utilization. Simply replace cell culture media with HypoThermosol® FRS and place plated cells into the cold for 1-3 days. Following storage, remove plates from cold, replace the HypoThermosol® FRS with culture media and place plates in TC incubator. After a recovery interval, the cells will be ready for utilization in any number of applications.

Phone: 1.425.402.1400 or toll-free: 1.866.424.6543
Fax: 1.425.402.1433
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BioLife Solutions, Inc.
3303 Monte Villa Parkway, Suite 310 | Bothell, WA 98021 USA
Web: www.biolifesolutions.com
E-mail: info@biolifesolutions.com

iii. Re-suspend as necessary in CryoStor® (for cryopreservation)
   a. Suspend cell pellet directly in cold (2-8°C) CryoStor.
   b. Transfer sample to cryovials.
   c. Pre-freeze: incubate samples at 2-8°C for 10 minutes.
   d. Freeze samples following standard protocol (1°C/min).
      1. Controlled rate freeze
         a) Nucleation: Freeze samples at -70°C (many protocols utilize -70°C and -80°C interchangeably).
         b) Use a controlled rate freeze (-1°C/min) or similar protocol for most mammalian cell systems.
         c) The freezing device or isopropanol container should be pre-cooled to 2-8°C.
         d) Ice nucleation within the sample (seeding) should be initiated at approximately -5°C using either a liquid nitrogen burst program setting on a controlled rate freezer or mechanical agitation (flick or tap) of the cryovial/sample container after approximately 15-20 min. at -70°C.
         e) Freeze time (-70°C) using isopropanol containers is recommended to be 3-4 hours.
   e. Storage: Place samples into storage
      1. Store samples at liquid nitrogen temperatures (below -130°C)
      2. Sample storage at -80°C is only recommended for short-term storage (weeks to months).
   f. Thaw
      1. Standard practice.
      2. Remove sample from liquid nitrogen and immediately place into 37°C H_2O bath for 2-4 minutes to warm samples until just thawed (cryovial should still feel cold).
      3. Gently agitate sample during the thawing interval to achieve uniform thawing of the sample.
      4. Once ice has melted, immediately transfer samples to a sterile environment and dilute in 37°C culture media (1:10 sample to culture media dilution ratio or greater) for cell culture.

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g. Testing
   1. Apply standard assays.
   2. Assessment immediately post-thaw tends to render incomplete and inaccurate data regarding sample viability and function; therefore, it is recommended that viability assessment is performed 24 to 48 hours post-thaw.
      a) Note: viability and yield assessment immediately following thawing may be helpful in evaluating the extent of delayed onset cell death (i.e. when comparing 1-hour post-thaw values to 24-hours post-thaw values).
      b) When determining preservation efficacy, make sure assessment is performed with careful consideration and comparison of both yields and viability between pre-freeze values, post-thaw values, and 24-48 hours post-thaw. This will allow for an accurate determination of sample status and preservation efficacy.

For questions regarding this protocol or immediate assistance,
please call BioLife Solutions Research and Technical Personnel 866-424-6543

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